UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/623,930	07/21/2003	Vicki Bowman Vance	USC-164	6465

22827 7590 04/16/2010

DORITY & MANNING, P.A.
POST OFFICE BOX 1449
GREENVILLE, SC 29602-1449

EXAMINER	
KUMAR, VINOD	
ART UNIT	**PAPER NUMBER**
1638	
MAIL DATE	**DELIVERY MODE**
04/16/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 10/623,930
Filing Date: July 21, 2003
Appellant(s): VANCE ET AL.

Christina L. Mangelsen
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed January 5, 2010 appealing from the Office action mailed June 2, 2009.

1. Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

2. Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

3. Status of Claims

The statement of the status of the claims contained in the brief is correct.

4. Status of Amendments

The appellant's statement of the status of amendments contained in the brief is correct. The appellant's statement that all amendments have been entered into the record is correct.

5. Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

6. Grounds of Rejection to be Reviewed on Appeal

Appellant's statement of the grounds of rejection to be reviewed on appeal is correct.

7. Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

8. Evidence Relied Upon

Cullen et al. (U.S. Published Patent Application No. 2004/0053411, claiming filing benefit of U.S. Provisional Patent Application 60/337,224 having filing date of May 3, 2002).

Llave et al. (The Plant Cell, 14:1605-1619, 2002).

Reinhart et al. (Genes and Development, 16:1616-1626, 2002).

9. Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

Claims 20, 23 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cullen et al. (US Patent Publication No. 2004/0053411, Published March 18, 2004, filed May 5, 2003, Provisional application filed May 3, 2002) in view of Llave et al. (The Plant Cell, 14:1605-1619, Published July 1, 2002) and Reinhart et al. (Genes and Development, 16:1616-1626, Published July 1, 2002).

Claims are drawn to a plant cell, plant or transformed seed of said plant comprising stably transformed with an miRNA precursor construct, said miRNA precursor construct comprising a promoter functional in a plant cell, wherein the promoter is operably linked to a nucleotide sequence encoding an isolated plant miRNA precursor, wherein the isolated plant miRNA precursor has been modified by (a) replacing an endogenous miRNA sequence of the isolated plant miRNA precursor with an exogenous miRNA sequence that maintains the length of the endogenous miRNA sequence; and (b) modifying nucleotides opposite the exogenous miRNA sequence in the isolated plant miRNA precursor to maintain double strandedness and mismatches of the isolated plant miRNA precursor, and further wherein the exogenous miRNA sequence is complementary to a target mRNA sequence within said plant and, following processing from said plant miRNA precursor, hybridizes with the target mRNA sequence, whereby the expression of the target sequence is reduced.

MicroRNAs (miRNAs) are a class of non-coding RNA gene whose final product is ~ 18-25 nt (nucleotides) long functional RNA molecule. They play important roles in the regulation of target genes by binding to complementary region of messenger transcripts (mRNAs) to repress their translation or regulate degradation. Thus miRNAs negatively regulate the expression of their complementary mRNAs at the post-transcriptional level. MicroRNAs (miRNAs) are produced from ~ 70-100 nt precursor (miRNA precursor) that forms a predicted RNA stem-loop structure. The stable secondary structure of miRNA precursor is predicted using bioinformatics based prediction tools, such as mfold. This information pertaining to structure and function of plant miRNAs, and the methods to determine stable secondary structure of miRNA precursors were known prior to the instantly claimed invention. The instant specification does not add anything new about structure and function of plant miRNAs that was not known prior to the claimed invention.

Cullen et al. teach designing an artifical miRNA precursor by modifying a naturally occurring miRNA precursor sequence with an exogenous miRNA to target and post-transcriptionally silence a gene of interest in a cell. The reference teaches that said modification comprises incorporating an miRNA sequence of interest into said miRNA precursor by substituting stem sequences of its native miRNA to generate miRNAs suitable for use in inhibiting expression of any target gene of interest in any host cell including a plant cell. The reference clearly teaches that bulges may be present in the sequence. The reference further teaches expressing said artifically designed miRNA precursor from a DNA expression vector in any host cell, including a plant cell. The reference also teaches that the modified or artificial miRNA precursor undergoes normal biogenesis to release non-native

miRNA which participates in post-transcriptional gene silencing of the target gene of interest. See in particular, paragraphs 0009; figures 1-8; paragraphs 0022, 0024-0027, 0029, 051-0053, 0057-0058.

Cullen et al. do not teach a plant miRNA precursor.

Llave et al. teach a number of plant miRNA precursors comprising an endogenous miRNA sequence. The reference also teaches that plant miRNA precursors contain short and simple stem-loop structures. The reference further teaches that plant miRNAs are small (predominately 21 to 24 nucleotides in length), arise by processing of miRNA precursor transcripts (~ 70 nucleotides) containing imperfectly paired stem structures in a Dicer-dependent manner. The reference further teaches cloning, sequencing and predicting secondary structures of said precursors which are capable of undergoing normal biogenesis to produce miRNA. The reference further teaches that plant miRNAs comprise a sequence which is complementary to a portion of an endogenous gene sequence whose expression is regulated by said miRNA sequence through perfect or nearly perfect binding to the endogenous target sequence. The reference also teaches a method of making a transgenic plant comprising transformation of a plant with a DNA construct comprising a mRNA inhibitory sequence (dsRNAi) operably linked to promoter functional in plant. See in particular, page 1605, abstract; page 1608, table 1; page 1609, figure 4; page 1611, table 2; page 1612, table 3; page 1613, figure 6; page 1614, figure 7; page 1617, sequence accession numbers.

Reinhart et al. teach plant miRNA precursors comprising an endogenous miRNA sequence which is released during the processing of the miRNA precursor to play a role in

post-transcriptional gene regulation of specific endogenous plant gene(s). The reference further teaches cloning, sequencing and predicting fold-back secondary structures (using RNAfold program) of said precursors which are capable of undergoing normal biogenesis to produce miRNA. Reinhart et al. also teach that said plant miRNA precursor comprises few mismatches in the miRNA sequence resulting in bulges. See in particular, abstract; page 1618, table 1; page 1619, figure 1; page 1622, figure 4.

At the time the invention was made, it would have been prima facie obvious and within the scope of an ordinary skill in the art to use the method of silencing the expression of a desired gene in a cell as taught by Cullen et al., to silence a desired gene in a plant or plant cell. It would have been obvious to use a recombinant DNA encoding a plant miRNA precursor sequence as taught by Llave et al. or Reinhart et al. and modify the plant miRNA precursor sequence by replacing the native miRNA sequence with an exogenous miRNA sequence which is complementary to a gene transcript of interest for down-regulating or silencing the expression of said desired gene in a plant cell or plant. One would have used any plant transformation vector and method to make the plant cell or plant, including the one taught by Llave et al.

Given that Llave et al. and Reinhart et al. teach that plant miRNA precursors comprising a native miRNA sequence which regulates the expression of specific plant gene, and Cullen et al. teach designing artifical (same as modified) miRNA precursor comprising incorporating an exogenous (non-native) miRNA sequence of interest into a naturally occurring miRNA precursor, one of ordinary skill in the art would have been motivated to modify a naturally occurring plant miRNA precursor by incorporating an exogenous miRNA sequence which is complementary to a target transcript of interest within the plant. One of

ordinary skill in the art would have been motivated to do so for the purpose of down-regulating the expression of any target gene of interest, depending on ones desired end.

Given that many native miRNA sequences contain mismatches or "bulges" as seen in Llave et al. and Reinhart et al., it would have been obvious to maintain the size, and positions of mismatches of the native miRNA secondary structure in the non-native miRNA sequence of the modified plant miRNA precursor, to avoid any possible problems during processing of the miRNA precursor. Thus it would have been obvious and within the scope of one of ordinary skill in the art to have arrived at the claimed plant cells or plant exhibiting reduced expression of the target gene with a reasonable expectation of success.

Given that Cullen et al. teach that transcribing a miRNA precursor from a vector in a plant cell host, opens up the possibility of long term stable gene-silencing of a target gene of interest, one of ordinary skill in the art would have been motivated to express said modified plant miRNA precursor sequence in a transgenic plant for the purpose of studying the function of a target gene of interest in growth and development for example, with a reasonable expectation of success. Obviously seeds would have also been produced for the purpose of propagation of said transgenic plants.

Thus, the claimed invention as a whole is prima facie obvious over the combined teachings of the prior art.

(10) Response to Arguments

Claims 20, 23 and 26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cullen et al. (US Patent Publication No. 2004/0053411, Published March 18, 2004, filed May 5, 2003, Provisional application filed May 3, 2002) in view of Llave et al. (The Plant Cell,

14:1605-1619, Published July 1, 2002) and Reinhart et al. (Genes and Development, 16:1616-1626, Published July 1, 2002). The rejection is maintained for the reasons of record.

Appellant traverses the rejection in the brief on appeal filed January 10, 2010.

Appellant traverses primarily that instantly claimed invention is patentable over Cullen et al., Llave et al. and Reinhart et al. because (1) Cullen et al., Llave et al. and Reinhart et al. fail to disclose or suggest instantly claimed elements, (2) Cullen et al. teach away from the instantly claimed invention, (3) Cullen et al., Llave et al., and Reinhart et al. fail to enable the instantly claimed invention, and (4) obviousness is based upon improper hindsight reasoning.

Traversal 1. Appellant argues that Cullen et al., Llave et al., and Reinhart et al. taken either alone or in any proper combination fail to disclose or suggest elements of independent claims 20 and 23. Appellant alleges that the designed miRNA precursor (mir-30-nxt precursor) of Cullen et al. do not maintain the length of the endogenous miRNA sequence. Appellant further alleges that Cullen et al. do not suggest that the designed precursor should maintain the length of the endogenous miRNA sequence. Appellant further alleges that neither Llave et al. nor Reinhart et al. teach designing an artificial miRNA precursor having an exogenous miRNA sequence. Appellant further argues that there is no suggestion in Cullen et al., Llave et al. or Reinhart et al. to maintain the length and secondary structure of an endogenous miRNA sequence following replacement with the exogenous miRNA sequence in an artificial miRNA construct. Appellant further argues that Cullen et al. teachings prefer that the stem comprises a perfectly complementary duplex. Appellant also argues that Cullen et al., Llave et al. or Reinhart et al. fail to teach or suggest an miRNA construct in which modification of the nucleotides opposite the exogenous miRNA sequence has been modified to maintain mismatches of the isolated precursor. Appellant also cites Vance's declaration

filed 1/3/2007 under 37 CFR § 1.1.32 to support the argument that the sequences opposite to the artificial miRNA in the stem of the miRNA precursor are modified to maintain the secondary structure of the stem so that artificial miRNA precursor is properly processed (brief on appeal, page 6, line 3 through line 2 of page 10).

Appellant's traversals are carefully considered but are deemed to be unpersuasive for the following reasons:

Contrary to Appellant's allegations, Cullen et al. do teach designing an artifical miRNA precursor by modifying a naturally occurring miRNA precursor sequence with an exogenous miRNA to target and post-transcriptionally silence a gene of interest in a cell. The reference teaches that said modification comprises incorporating an miRNA sequence of interest into said miRNA precursor by substituting stem sequences of its native miRNA to generate miRNAs suitable for use in inhibiting expression of any target gene of interest in any host cell including a plant cell. The reference clearly teaches that bulges may be present in the sequence. The reference further teaches expressing said artifically designed miRNA precursor from a DNA expression vector in any host cell, including a plant cell. The reference also teaches that the modified or artificial miRNA precursor undergoes normal biogenesis to release non-native miRNA which participates in post-transcriptional gene silencing of the target gene of interest. See US Patent Publication No. 2004/0053411 at paragraphs 0009; figures 1-8; paragraphs 0022, 0024-0027, 0029, 051-0053, 0057-0058.

Appellant's attention is also drawn to paragraphs 0022 and 0023 of Cullen et al., wherein the reference clearly teach that it was well known in the prior art how to design stem-loop structure in an artificial miRNA precursor such that it is recognized by a ribonuclease (e.g., an RNAse 111-type enzyme, such as DICER, or an enzyme having the recognition

properties thereof), with the resulting excision of the mature miRNA. For example, the precursor stem-loop structures can be about 40 to 100 nucleotides long. The stem region can be about 19-45 nucleotides or more in length. The stem can comprise a perfectly complementary duplex, however, "bulges" can be present on either arm of the stem. Such "bulges" may be few in number (e.g., 1, 2 or 3) and are about 3 nucleotides or less in size.

Appellant's argument is not persuasive to suggest that maintaining mismatches (bulges) present in an endogenous miRNA sequence, following replacement with an exogenous miRNA sequence in an artificial miRNA precursor construct is absolutely important for efficient miRNA processing from the artificial miRNA precursor. See for example figures 2-3 of Cullen et al., wherein mi-30-nxt precursor which lacked mismatches (bulges) in its miRNA, compared to endogenous mir-30 precursor, was capable of processing efficiently to release its miRNA (nxt) sequence and thereby reducing the target gene expression. Appellant's attention is also drawn to Niu et al. (Nature Biotechnology, 24:1420-2331, 2006; cited in Vance's declaration filed on 1/3/2007 under 37 CFR § 1.132) who teach that a plant pre-amiRNA159 precursor designed to contain amiRNA sequence which was fully complementary to the amiRNA* (strand opposite to amiRNA) sequence, was capable of undergoing efficient processing to release amiRNA to confer virus resistance in *Arabidopsis* plants (see in particular, page 1421, figure 1; page 1421, figure 2).

In response to Appellant's argument that Cullen et al. do not teach maintaining the secondary structure of the modified plant miRNA precursor, it is maintained that it would have been obvious and within the scope of an ordinary skill in the art to maintain the secondary structure of the modified plant miRNA precursor because it was unknown what the effect of

removing the secondary structure would be, it would have been obvious not to alter it. Since known naturally occurring plant miRNA precursors have mismatches (bulges) in the miRNA* (the nucleotide sequence opposite the miRNA), it would have been obvious and within the scope of one of ordinary skill in the art to maintain said bulges in the modified miRNA precusor to preserve the secondary structure and free energy exactly the same as that of a naturally occurring miRNA precursor. One of ordinary skill in the art would have been motivated to do so for the purpose of avoiding any possible problems during processing of the miRNA precursor. It is also noted that that the secondary structure of the modified miRNA precursor would have been maintained, if the modified miRNA precusor was derived from a naturally occurring miRNA precursor lacking bulges (mismatches) in miRNA* (strand opposite miRNA). That is, if the miRNA and miRNA* in the naturally occuring miRNA precursor were fully complementary, there would be no need to make modifications to preserve the positions of bulges (mismatches) in the modified miRNA precursor.

Traversal 2. Appellant argues that Cullen et al. teach away from the claimed invention. Appellant alleges that based on Cullen et al. teachings, one of skill in the art would infer that forming an artifical miRNA precursor construct, the maintenance of the length of endogenous miRNA sequence in the exogenous miRNA sequence of the artifical miRNA precursor, and maintenance of the secondary structure of the miRNA precursor are not necessary. Appellant further argues that since Office action mailed October 31, 2006 had raised the issue of secondary structure by emphasizing its importance in designing artifical miRNA precursors, and thus Cullen et al. teachings would have taught one of ordinary skill in

the art away from the claimed invention (brief on appeal, page 10, line 16 through page 13, line 15).

Appellant's traversals are carefully considered but are deemed to be unpersuasive for the following reasons:

Appellant's attention is drawn to paragraphs 0022 and 0023 of Cullen et al., wherein the reference clearly provides options for designing an artificial miRNA precursor that is recognized by a ribonuclease so that the artificial precursor is efficiently processed to release exogenous miRNA sequence to inhibit the expression of a target gene of interest. For example, the precursor stem-loop structures can be about 40 to 100 nucleotides long. The stem region can be about 19-45 nucleotides or more in length. The stem can comprise a perfectly complementary duplex, however, "bulges" can be present on either arm of the stem. Such "bulges" may be few in number (e.g., 1, 2 or 3) and are about 3 nucleotides or less in size. It is noted that Appellant is mischaracterizing the teachings of Cullen et al. Cullen et al. do not suggest that a stable secondary structure is unimportant for the normal biogenesis of miRNA precursor to release miRNA. Contrary to Appellant's allegations, Cullen et al. clearly teach that it was well known in the prior art how to design a stable stem-loop structure in a miRNA precursor so that the precursor is efficiently processed to release miRNA. Cullen et al. even cite prior art references to support these teachings (see lines 3-4 of paragraph 0022 of Cullen et al.).

There is no doubt that a stable secondary structure is essential for efficient processing of a miRNA precursor to release miRNA. This was well known in the prior art as discussed above and further admitted by Appellant through Vance's declaration filed 1/3/2007 under 37

CFR § 1.1.32. However, Appellant's argument is not persuasive to suggest that maintaining mismatches (bulges) present in an endogenous miRNA sequence, following replacement with an exogenous miRNA sequence in an artificial miRNA precursor construct is absolutely important for maintaining stable secondary structure of miRNA precursor for efficient miRNA processing from the artificial miRNA precursor. For example, Cullen et al. mi-30-nxt precursor which lacked mismatches (bulges) in its miRNA, compared to endogenous mir-30 precursor, was capable of processing efficiently to release its miRNA (nxt) sequence and thereby reducing the target gene expression. More specifically, Niu et al. (Nature Biotechnology, 24:1420-2331, 2006; cited in Vance's declaration filed 1/3/2007 under 37 CFR § 1.132; see page 5, lines 20-29 of the declaration) teach that a plant pre-amiRNA159 precursor designed to contain amiRNA sequence which was fully complementary to the amiRNA* (strand opposite to amiRNA) sequence, was capable of undergoing efficient processing to release amiRNA to confer virus resistance in *Arabidopsis* plants (see in particular, page 1421, figure 1; page 1421, figure 2).

In response to Appellant's argument that Office action mailed October 31, 2006 had raised the issue of secondary structure by emphasizing its importance in designing artifical miRNA precursors, it is noted that Office raised this issue in a separate rejection under 35 USC 112, first paragraph (now withdrawn) because the specfification had provided prophetic guidance on the design of an artifical miRNA precusor. However, Appellant's declarations filed under 37 CFR § 1.1.32 (see Vance's declaration filed 1/3/2007; and Bowman's declaration filed 8/20/2007) clearly suggested that bioinformatics based tools were available in the prior art to determine how to design an artifical stem-loop structure with a stable

secondary structure. This implies that Cullen et al., and Appellant's declarations clearly suggest that it was well known in the prior art how to design a stable stem-loop structure in an artifical miRNA precursor so that it is efficiently processed to release miRNA. Thus, Appelllant's argument is not persusaive that Cullen et al. teach away from the instantly claimed invention.

Traversal 3. Cullen et al., Llave et al., and Reinhart et al. fail to enable the instantly claimed invention.

Appellant further argues that Cullen et al., Llave et al., and Reinhart et al. taken alone or in any proper combination fail to enable the claimed invention. Appellant while admitting that Cullen et al. do teach expressing artificial miRNA precursors to inhibit the expression of a target gene in plant cells, however, alleges that Cullen et al. do not teach any plant miRNAs or miRNA precursors. Appellant also alleges that Office had previously acknowledged (Office action of October 31, 2006) that differences exist in structure and production of miRNA precursors from diverse sources, and thus one of ordinary skill in the art would have concluded that artificial miRNA precursor construct derived from plant source would be different from the one derived from animal source. Appellant further alleges that no examples of artificial nucleic acid constructs including plant miRNA precursors are taught. Appellant further argues that Cullen et al. teach an animal-based construct that does not maintain the length or secondary structure of the endogenous precursor, and provide no specific teachings to the formation of transformed plants (brief on appeal, page 13, line 16 through line 15 of page 15).

Appellant's traversals are carefully considered but are deemed to be unpersuasive for the following reasons:

Contrary to Appellant's allegations, Cullen et al. clearly teach that it was well known in the prior art how to design a stable stem-loop structure in a miRNA precursor so that the precursor is efficiently processed to release miRNA. Cullen et al. even cite prior art references to support these teachings (see lines 3-4 of paragraph 0022). Appellant's attention is also drawn to paragraphs 0022 and 0023 of Cullen et al., wherein the reference clearly provides choices for designing an artificial miRNA precursor that is recognized by a ribonuclease so that precursor is efficiently processed to release exogenous miRNA sequence to inhibit the expression of a target gene of interest. For example, the precursor stem-loop structures can be about 40 to 100 nucleotides long. The stem region can be about 19-45 nucleotides or more in length. The stem can comprise a perfectly complementary duplex, however, "bulges" can be present on either arm of the stem. Such "bulges" may be few in number (e.g., 1, 2 or 3) and are about 3 nucleotides or less in size. Contrary to Appellant's allegations, Cullen et al. do not suggest that a stable secondary structure is unimportant for the normal biogenesis of miRNA precursor to release miRNA. Additionally, Appellant has clearly admitted through the declaration of Vance filed 1/3/2007 under 37 CFR § 1.1.32 (see pages 2-6) that bioinformatics based tools were available in the prior art to determine how to design an artifical stem-loop structure with a stable secondary structure.

Appellant's argument is not persuasive to suggest that maintaining mismatches (bulges) present in an endogenous miRNA sequence, following replacement with an exogenous miRNA sequence in an artificial miRNA precursor construct is absolutely

important for maintaining stable secondary structure of miRNA precursor for efficient miRNA processing from the artificial miRNA precursor. This is evident through Cullen et al. teachings that mi-30-nxt precursor which lacked mismatches (bulges) in its miRNA, compared to endogenous mir-30 precursor, was capable of processing efficiently to release its miRNA (nxt) sequence and thereby reducing the target gene expression (see in particular, figures 2 and 3). Appellant's attention is also drawn to page 1421, figure 1; page 1421, and figure 2 of Niu et al. (Nature Biotechnology, 24:1420-2331, 2006; cited in Vance's declaration filed on 1/3/2007 under 37 CFR § 1.132; see page 5, lines 20-29 of the declaration), wherein the reference teach that a plant pre-amiRNA159 precursor designed to contain amiRNA sequence which was fully complementary to the amiRNA* (strand opposite to amiRNA) sequence, was capable of undergoing efficient processing to release amiRNA to confer virus resistance in *Arabidopsis* plants.

While it is understood that Cullen et al. do not specifically teach the structure of a plant miRNA precursor, however, this deficiency is overcome by the teachings of Llave et al. and Reinhart et al. Appellant's attention is drawn to page 1605, abstract; page 1608, table 1; page 1609, figure 4; page 1611, table 2; page 1612, table 3; page 1613, figure 6; page 1614, figure 7; page 1617, and sequence accession numbers of Llave et al., wherein Llave et al. teach a number of plant miRNA precursors comprising an endogenous miRNA sequence. Llave et al. also teach that plant miRNA precursors contain short and simple stem-loop structures. The reference further teaches that plant miRNAs are small (predominately 21 to 24 nucleotides in length), arise by processing of miRNA precursor transcripts (~ 70 nucleotides) containing imperfectly paired stem structures in a Dicer-dependent manner. The

reference further teaches cloning, sequencing and predicting secondary structures of said precursors which are capable of undergoing normal biogenesis to produce miRNA. The reference further teaches that plant miRNAs comprise a sequence which is complementary to a portion of an endogenous gene sequence whose expression is regulated by said miRNA sequence through perfect or nearly perfect binding to the endogenous target sequence. The reference also teaches a method of making a transgenic plant comprising transformation of a plant with a DNA construct comprising a mRNA inhibitory sequence (dsRNAi) operably linked to a promoter functional in a plant.

Appellant's attention is also drawn to abstract; page 1618, table 1; page 1619, figure 1; page 1622, and figure 4 of Reinhart et al., wherein the reference teach plant miRNA precursors comprising an endogenous miRNA sequence which is released during the processing of the miRNA precursor to play a role in post-transcriptional gene regulation of specific endogenous plant gene(s). The reference further teaches cloning, sequencing and predicting fold-back secondary structures (using RNAfold program) of said precursors which are capable of undergoing normal biogenesis to produce miRNA. Reinhart et al. also teach that said plant miRNA precursor comprises few mismatches in the miRNA sequence resulting in bulges.

Given (a) Cullen et al. clearly teach expressing an artifical miRNA precursor in a plant cell to inhibit expression of a target gene of interest, (b) Llave et al. and Reinhart et al., teach structure and function of plant miRNA precursors, including a method of obtaining a transgenic plant, and (c) Cullen et al. teach that it was well known in the prior art how to design a stable stem-loop structure in a miRNA precursor so that the precursor is efficiently processed to release miRNA (also admitted by Appellant in Vance's declaration filed on

1/3/2007 under 37 CFR § 1.132; emphasis added), it would have been obvious and within the scope of an ordinary skill in the art to have combined the teachings of Cullen et al., Llave et al. and Reinhart et al. as discussed above to arrive at the claimed invention with a reasonable expectation of success. In view of this, Appellant's arguments are not persuasive to suggest that teachings of Cullen et al., Llave et al., and Reinhart et al. fail to enable the instantly claimed invention. This is especially important since Appellant's specification provides a prophetic guidance (no working example) on instantly claimed invention and the prophetic guidance relies entirely on what was known in the prior art about plant miRNA precursor biogenesis to release miRNA to inhibit the expression of a gene of interest. This is further evident from Vance's declaration filed on 1/3/2007 under 37 CFR § 1.132.

It is, therefore, maintained that at the time the invention was made, it would have been prima facie obvious to one of ordinary skill in the art to use the method of silencing the expression of a desired gene in a cell as taught by Cullen et al., to silence a desired gene in a plant or plant cell. It would have been obvious to use a recombinant DNA encoding a plant miRNA precursor sequence as taught by Llave et al. or Reinhart et al. and modify the plant miRNA precursor sequence by replacing the native miRNA sequence with an exogenous miRNA sequence which is complementary to a gene transcript of interest for down-regulating or silencing the expression of said desired gene in a plant cell or plant. One would have used any plant transformation vector and method to make the plant cell or plant, including the one taught by Llave et al.

Given that Llave et al. and Reinhart et al. teach that a plant miRNA precursor comprising a native miRNA sequence which regulates the expression of a specific plant gene, and Cullen et al. teach designing artificial (same as modified) miRNA precursors

comprising incorporating an exogenous (non-native) miRNA sequence of interest into a naturally occurring miRNA precursor, one of ordinary skill in the art would have been motivated to modify a naturally occurring plant miRNA precursor by incorporating an exogenous miRNA sequence which is complementary to a target transcript of interest within the plant. One of ordinary skill in the art would have been motivated to do so for the purpose of down-regulating the expression of any target gene of interest, depending on one's desired end.

Given that many native miRNA sequences contain mismatches or "bulges" as seen in Llave et al. and Reinhart et al., it would have been obvious to maintain the size, and positions of mismatches of the native miRNA secondary structure in the non-native miRNA sequence of the modified plant miRNA precursor, to avoid any possible problems during processing of the miRNA precursor. Thus it would have been obvious and within the scope of one of ordinary skill in the art to have arrived at the claimed plant cells or plant exhibiting reduced expression of the target gene with a reasonable expectation of success.

Given that Cullen et al. teach that transcribing a miRNA precursor from a vector in a plant cell host opens up the possibility of long term stable gene-silencing of a target gene of interest, one of ordinary skill in the art would have been motivated to express said modified plant miRNA precursor sequence in a transgenic plant for the purpose of studying the function of a target gene of interest in growth and development for example, with a reasonable expectation of success. Obviously seeds would have also been produced for the purpose of propagation of said transgenic plants.

Traversal 4. Obviousness is based upon improper hindsight reasoning.

Appellant argues primarily that only improper hindsight gained from Appellant's disclosure would lead the person of ordinary skill to arrive at the instantly claimed invention (page 15, line 16 through line 4 page 16).

Appellant's traversals are carefully considered but are deemed to be unpersuasive for the following reasons:

Appellant's argument is not persuasive to suggest that examiner's conclusion of obviousness is based upon improper hindsight reasoning, because it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). In the instant case, Cullen et al. teachings clearly suggest that it was well known in the prior art how to design a stable stem-loop structure in a miRNA precursor so that the precursor is efficiently processed to release miRNA. Cullen et al. even cite prior art references to support these teachings (see lines 3-4 of paragraph 0022 of Cullen et al). Appellant's attention is also drawn to paragraphs 0022 and 0023 of Cullen et al., wherein the reference clearly provides choices for designing an artificial miRNA precursor that is recognized by a ribonuclease so that precursor is efficiently processed to release exogenous miRNA sequence to inhibit the expression of a target gene of interest. For example, the precursor stem-loop structures can be about 40 to 100 nucleotides long. The stem region can be about 19-45 nucleotides or more in length. The stem can comprise a perfectly complementary duplex, however, "bulges" can be present

on either arm of the stem. Such "bulges" may be few in number (e.g., 1, 2 or 3) and are about 3 nucleotides or less in size.

Contrary to Appellant's allegations, Cullen et al. do not suggest that a stable secondary structure is unimportant for the normal biogenesis of miRNA precursor to release miRNA. Appellant's attention is drawn to Vance's declaration filed 1/3/2007 under 37 CFR § 1.1.32 (see pages 2-6) which states that bioinformatics based tools were available in the prior art to determine how to design an artifical stem-loop structure with a stable secondary structure.

It is also important to note that maintaining mismatches (bulges) present in an endogenous miRNA sequence, following replacement with an exogenous miRNA sequence in an artificial miRNA precursor construct is not important for maintaining stable secondary structure of miRNA precursor for efficient miRNA processing from the artificial miRNA precursor. This is evident from the teachings of Cullen et al., wherein the reference teach mi-30-nxt precursor which lacked mismatches (bulges) in its miRNA, compared to endogenous mir-30 precursor, was capable of processing efficiently to release its miRNA (nxt) sequence and thereby reducing the target gene expression. This is also evident through Niu et al. (Nature Biotechnology, 24:1420-2331, 2006; cited in Vance's declaration filed 1/3/2007 under 37 CFR § 1.1.32; see page 5, lines 20-29 of the declaration) teachings, wherein the reference teaches that a plant pre-amiRNA159 precursor designed to contain amiRNA sequence which was fully complementary to the amiRNA* (strand opposite to amiRNA) sequence, was capable of undergoing efficient processing to release amiRNA to confer virus resistance in *Arabidopsis* plants (see in particular, page 1421, figure 1; page 1421, figure 2).

This implies that while it is understood that a stable secondary structure is essential for efficient processing of a miRNA precursor to release miRNA as stated in the prior art cited in Cullen et al., and further admitted by Appellant in Vance's declaration filed 1/3/2007 under 37 CFR § 1.1.32, however, maintaining mismatches (bulges) present in an endogenous miRNA sequence, following replacement with an exogenous miRNA sequence in an artificial miRNA precursor construct is not absolutely important for maintaining stable secondary structure of miRNA precursor for efficient miRNA processing from the artificial miRNA precursor.

Thus, the claimed invention as a whole is prima facie obvious over the combined teachings of the prior art.

11. Related Proceedings Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejection should be sustained.

Respectfully submitted,

/Vinod Kumar/

Art Unit 1638

Conferees:

/Vinod Kumar/

Primary Examiner

Art Unit 1638

/Anne Marie Grunberg/

Supervisory Patent Examiner

Art Unit 1638

/Joseph T. Woitach/

Supervisory Patent Examiner

Art Unit 1633